COMPETING INTERESTS STATEMENT
The authors declare no competing financial interests.

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The ABCs of low-phytate crops

Victor Raboy

The nutritional quality of maize and soybean seeds is improved by embryo-specific silencing of an ABC transporter.

Adequate dietary phosphorus is essential for human health and optimal livestock production. Although cereal and legume seeds contain plenty of phosphorus, most is in the form of phytic acid (inositol hexaphosphate), which nonruminants cannot digest efficiently. In this issue, Shi and colleagues[1] describe a new strategy for increasing the available phosphorus content of seeds. They identify an ATP-binding cassette (ABC) transporter as a key contributor to phytic-acid accumulation in maize and soybean seeds and then silence it to engineer a dominant, seed-specific block in phytic-acid accumulation. Besides revealing a likely role for subcellular compartmentation in controlling cellular phytic-acid levels, this study has important nutritional and environmental implications.

Phytic acid is ubiquitous in eukaryotes and regulates many cellular functions, including stress responses, development, phosphate sensing and homeostasis, DNA repair, RNA editing and mRNA export[2]. The biosynthesis of phytic acid is largely cytoplasmic and begins with the synthesis of phytic acid's backbone, *myo*-inositol and its phosphorylation (**Fig. 1**). In seeds, phytic acid is deposited as a mixed salt in inclusions within protein-storage vacuoles. Several enzymes important for the synthesis of phytic acid have been identified, along with the genes that encode them[3–6] (**Fig. 1**).

A reduction in the phytic-acid content of grains could have significant beneficial effects on human health. Besides sequestering inorganic phosphate, phytic acid chelates divalent cations, such as Fe^{2+}, Mn^{2+}, Mg^{2+}, Zn^{2+} and Ca^{2+}. In the developing world, the phytic-acid content of diets based on grains and oilseeds contributes to iron and zinc deficiencies that affect hundreds of millions of people today[7].

In developed countries, low–phytic acid grains could have both nutritional and environmental benefits in animal agriculture. Commercially raised monogastric animals—poultry, swine and fish—discharge phytate-derived phosphate-rich waste that contributes to water pollution by eutrophication[8]. To meet the nutritional needs of pigs and chickens, farmers often supplement animal feed either with an available form of phosphorus derived from rock phosphate or with phytase[9], which degrades feed phytate after ingestion to release phosphorus for uptake. However, as supplementation is costly, an attractive alternative is to deal with the problem at its source by developing low-phytate crops.

Starting in the early 1990s, several low–phytic acid (*lpa*) mutants were isolated in maize, barley, rice, wheat, soybean and *Arabidopsis thaliana*[3,4,10,11]. In seeds produced by plants homozygous for a given *lpa* allele, reductions in seed phytic acid of 50–95% are almost always accompanied by increases in inorganic phosphorus that maintain total seed phosphorus levels. Animal nutrition studies have confirmed that *lpa* seeds provide more available phosphorus and can reduce levels of phosphorus in animal waste[10]. Moreover, *lpa* seeds enhance iron, zinc and calcium nutrition in animals and humans[10].

The difficulty with these strategies, however, is that systemic reductions of phytic-acid levels usually have negative effects on seed and plant performance, such as compromised germination, emergence, stress tolerance and seed filling. Shi and colleagues have developed a more targeted approach that avoids systemic disruption of phytic-acid accumulation. Using map-based cloning, they identify a locus encoding an ABC transporter as the site of mutations that confer *lpa1* phenotypes in maize. As northern blot analysis indicates that the gene is expressed primarily in embryos, but also in other tissues within both the seed and vegetative organs, the authors introduce gene-silencing constructs under the control of embryo-specific promoters from either the oleosin or globulin-1 genes to minimize the effects of disrupting inositol phosphate metabolism in other tissues subsequent to seed development.

Gene suppression driven by either promoter recapitulates *lpa1* mutant phenotypes in terms of reduced phytate content and increased available phosphate and without significant decreases in seed dry weights. Although silencing by the globulin-1 construct is weaker than that of the oleosin construct, comparison of several lines from both classes of transformant leads the authors to conclude that the globulin-1 promoter is the better of the two for reducing seed phytate without reducing seed dry weight and impairing germination. The demonstration that the same effects can be seen in soybeans when the homologous soybean ABC transporter is specifically silenced in embryos using a trypsin-inhibitor promoter suggests that this approach should be applicable in many crops.

Although any of the enzymes shown in **Figure 1** might be engineered for seed- or tissue-specific regulation of phytic-acid synthesis, the authors argue that targeting the *lpa1* ABC transporter may be advantageous in that it avoids interference with metabolic pathways important to many other functions. This hypothesis should be further explored by more thorough assessments of seed development, dry weight accumulation, germination and seedling function to follow-up on the evaluation of seed weight and yield in the present report. An attractive alternative approach to engineering the low-phytate trait would be to overexpress sequences encoding a phytase[12] in developing seeds. In theory, use of such seed in feeds would provide high available phosphorus/low-phytate phosphorus and have the added advantage of also providing an active phytase that upon ingestion would break down phytates from other feed components.

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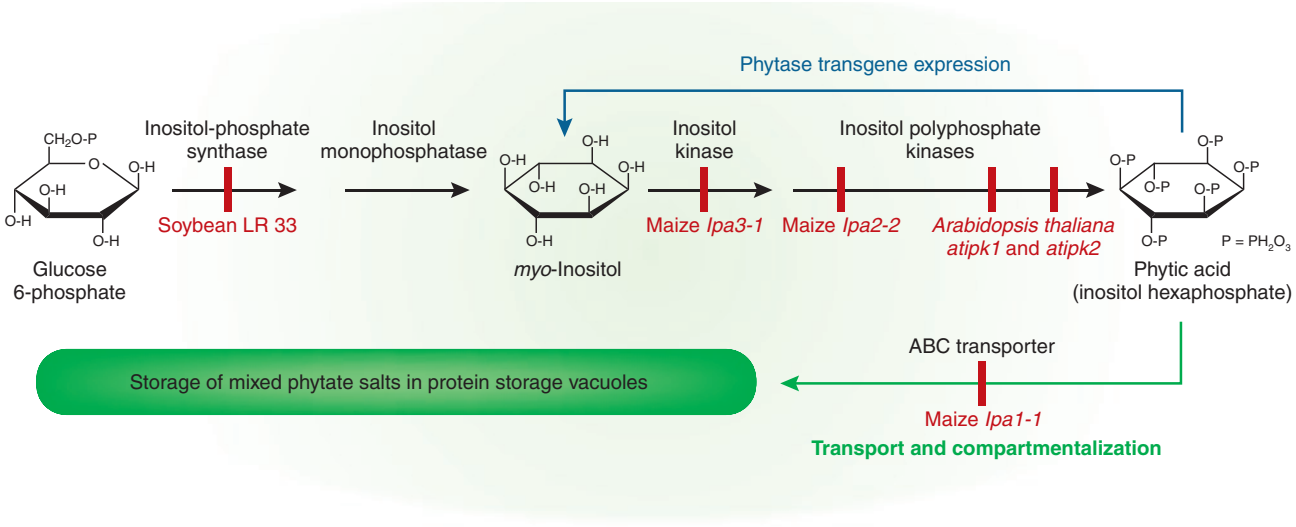

Figure 1 Potential targets for reducing phytic-acid levels during seed development. Phytic acid is believed to be synthesized primarily in the cytoplasm and is stored in protein storage vacuoles. Mutations that block phytic-acid synthesis or accumulation are indicated in red. Transgenic approaches involving expression of a phytase transgene to degrade phytic acid are shown in blue. Shi and colleagues[1] reduce the phytic-acid content of seeds by transgene-mediated silencing expression of an ABC transporter encoded by the maize *Ipa1* locus. The specific cargo and subcellular location of the transporter remain to be determined.

The significance of the study extends beyond its implications for agricultural biotechnology as it is the first report of a transport or compartmentalization function related to phytic-acid accumulation. Presumably, the ABC transporter regulates synthesis or storage of phytic acid. Although the subcellular location of the maize and soybean ABC transporters and the specific metabolite(s) it pumps have yet to be determined, the potential benefits of an effective approach to engineering low-phytate crops are clear. These include improving human nutrition, more efficient use of an expensive and limiting mineral supplement in animal feed and reducing a negative environmental consequence of commercial farming.

COMPETING INTERESTS STATEMENT
The author declares competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturebiotechnology/.

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Antibodies cut down to size

Robert Charles Ladner

Peptides only 28 amino acids long retain the specificity of a parental antibody.

Monoclonal antibodies provide unparalleled specificity for targeted drug delivery *in vivo*, but their efficacy against cancer may be limited by their large size, which prevents them from penetrating into solid tumors. As described in this issue, Qiu and colleagues[1] have whittled bulky antibodies to ~3 kDa, about 1/50 of their normal size. Moreover, they showed that the peptides can deliver a conjugated cytotoxin to tumors with high specificity and that the peptide-toxin fusions penetrate further into the tumor tissue than the parental antibodies. These new antibody mimetics, which have no disulfides and can be produced in *Escherichia coli*, represent a promising format for researchers interested in developing specific binders for a range of applications.

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Antibodies have six hypervariable loops called complementarity-determining regions (CDRs)—three in the heavy chain and three in the light chain—which determine their specificity. On each chain, the CDRs are interspersed with four framework regions (FRs) that maintain the CDRs in their proper orientations. Knowledge of the contacts made between the various CDRs to ensure antigen-binding (**Fig. 1**) led Qiu and colleagues to propose that two CDRs alone—one from the heavy chain and one from the light chain—might retain antigen specificity if separated by an FR that allows them to assume a conformation similar to that of the parental antibody after antigen binding.

Starting with the CDR sequences of HB-168, a monoclonal antibody against an envelope glycoprotein of Epstein Barr virus